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# MOLECULAR DIAGNOSTICS IN ONCOLOGY: NEW TRENDS

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Molecular diagnostics is a mandatory component of modern clinical oncology. The most known examples of molecular diagnostic procedures include the detection of hereditary cancer syndromes and the analysis of somatic drug-sensitizing mutations in protein kinases. Advances in cancer research as well as the development of new technologies led to emergence of new trends in this area of medicine. The invention of next generation sequencing (NGS) has a potential to dramatically change the landscape of molecular diagnostics. NGS allows to significantly improve the efficiency and availability of genetic testing for hereditary cancers as well as to undertake comprehensive tumor mutation profiling to guide the therapy choice. Tumors usually change their properties during therapeutic intervention. Monitoring of these properties is important for proper selection of further treatment options. So-called "liquid biopsy" is essential for this purpose, as it allows to detect key molecular features of the tumors by a non-invasive approach. There is an increasing popularity of *ex vivo* tumor models, which allow to cultivate tumor cells and to select the therapy based on the results of drug sensitivity tests.

**Keywords:** molecular diagnostics; oncology; heredity.

# МОЛЕКУЛЯРНАЯ ДИАГНОСТИКА В ОНКОЛОГИИ: НОВЫЕ ТЕНДЕНЦИИ

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Молекулярная диагностика стала неотъемлемым компонентом современной клинической онкологии. К наиболее известным направлениям этой области медицины относятся усилия, направленные на диагностику наследственных опухолевых синдромов, а также выявление соматических мутаций, ассоциированных с чувствительностью новообразований к ингибиторам протеинкиназ. Развитие знаний о механизмах развития неоплазм, а также создание новых технологий формируют новые тенденции в молекулярной диагностике рака. Наиболее заметным явлением как в биомедицине вообще, так и в онкологии в частности стало внедрение секвенирования нового поколения (next generation sequencing, NGS). Использование NGS позволяет многократно повысить эффективность и доступность диагностики наследственного рака, а также выполнять мутационное профилирование опухолей с целью персонализированного подбора терапии. Опухоли значительно видоизменяют свои свойства в процессе лечения, поэтому мониторинг биологического портрета новообразования представляет крайне важную задачу. В зависимости от результатов мониторинга и динамики молекулярного портрета трансформированных клеток появляется возможность назначения новых лекарственных препаратов. Важным инструментом в этом отношении является так называемая жидкостная биопсия, позволяющая анализировать существенные характеристики опухоли без применения инвазивных процедур. Большую популярность получили персонализированные *ex vivo* модели карцином. Они подразумевают культивирование опухолевых клеток и выполнение тестов на лекарственную чувствительность с целью индивидуального подбора противоопухолевой терапии.

**Ключевые слова:** молекулярная диагностика; онкология; наследственность.

**List of abbreviations**

NGS — next generation sequencing; EGFR — epidermal growth factor receptor.

## Introduction

Oncology is one of the main research areas for testing and implementing molecular medicine methods. There are several factors that determine the relevance of translational research in the field of cancer diagnosis and treatment. Firstly, malignant tumors represent one of a few human pathologies that almost always develop as a result of the accumulation of somatic mutations. Accordingly, these mutations serve as an object for basic research and as a target for medical interventions. Secondly, the diagnosis of a malignant neoplasm in itself implies verification by a morphologist. Thus, specialists have at their disposal the biological material of tumors obtained from each oncological patient. Such accessibility of pathologically altered tissues is a unique characteristic of oncology that provides high information content for research in this field of medicine. Thirdly, family tumor syndromes occupy the leading positions on the list of hereditary human diseases. Progress in medical genetics is largely determined by successes in studying predisposition to cancer diseases. Finally, it should be recognized that the word "cancer" is associated with an unfavorable prognosis of the disease, and undoubtedly, even taking into account all the ethical aspects of medical activity, the scope for using experimental approaches in oncology is somewhat broader than that in other fields of clinical medicine [1–4].

Molecular diagnostics represents an integral component of the examination of oncological patients. The formation of a system of medical actions for patients with hereditary cancer is the most notable practical success in molecular oncology. The genes responsible for the primary family tumor syndromes, such as hereditary breast and ovarian cancer, hereditary colon and endometrial cancer (Lynch syndrome), hereditary colon polyposis, Li-Fraumeni syndrome, were discovered back in the mid–1990s. This success of medical genetics has enabled the organization of effective diagnostics for familial tumor syndromes. There are methods to detect healthy carriers of "cancerous" mutations that are associated with a fatal predisposition to a particular type of neoplasm. Accordingly, measures have been developed for the early diagnosis of cancer in risk groups, and the ideology of preventive operations was formed. The unusual range of the drug sensitivity of hereditary tumors was reported at the end of the last decade. Currently, a large variety of therapeutic agents have been developed that can effectively treat some types of hereditary neoplasms of the breast, ovary, prostate, colon, thyroid gland, brain, etc. [4, 5]. A breakthrough in translational oncology was the almost random discovery of mutations associated with a change in the conformation of protein kinases and, as a result, with the selective sensitivity of mutated oncoproteins to individual drugs. Currently, practical oncology routinely applies tests aimed at analyzing mutations in the following genes: *EGFR* (epidermal growth factor receptor), *ALK*, *ROS1*, *BRAF*, *NTRK1*, *NTRK2*, *NTRK3*, *MET*, etc. Using targeted drugs specific for the listed kinases, the treatment results of certain categories of patients with lung cancer, melanoma, and some other tumors have been significantly improved. In general, the diagnostics of somatic mutations in neoplasms can be considered a separate component of examinations in cancer patients [4]. The achievements listed above have been successfully applied in clinical oncology, and they have already become the standards of medical care in almost all countries throughout the world. This review aims to present new trends in the molecular diagnosis of cancer, which are likely to become the main avenues of translational oncology diagnostics in the upcoming decade.

## Next-generation sequencing

The development and implementation of next generation sequencing (NGS) represents one of the main achievements of biomedical science over recent decades. The essence of NGS is the repeated reading of random fragments of the DNA matrix. Subsequent computer assembly of the fragments analyzed helps to recreate the original DNA sequence that was analyzed. A unique characteristic of NGS is its enormous productivity; for example, standard equipment for NGS can "read" the complete human genome within a few days [6, 7]. Furthermore, NGS can both analyze individual genomes and perform integrative analytical procedures. In particular, NGS is used for the individual characterization of microorganisms that inhabit the intestine [8, 9].

With multiple readings, NGS is extraordinarily sensitive; thus, this method can identify single mutated copies of genes in the environment of an excess of normal DNA sequences to detect trace amounts of tumor cells [10]. The most obvious application of NGS is the analysis of the human genome to diagnose hereditary diseases [11]. NGS has become a powerful tool for identifying new genes associated with hereditary pathologies. A complete analysis of the genome coding part, so-called whole-exome sequencing, is an integral component of the examination of patients with signs of genetic abnormalities in whom the cause of disease could not be established by standard laboratory analysis methods.

Although whole-exome sequencing seems to be the preferred method for detecting new genomic abnormalities, the sequencing of multi-genic panels is more popular in practical medicine, specifically for research. In particular, a huge number of hereditary diseases are characterized by the presence of phenocopies. This term describes the phenomenon when the same phenotype can be caused by mutations in different genes. A few years ago, a genetic examination of patients with similar diseases included a sequential analysis of all the genes involved in the suspected pathology. Such a procedure took several months and was characterized by an unusually high cost. The use of an NGS panel, which includes all potential candidate genes, is currently more justified. Equipment for NGS provides the possibility of the simultaneous examination of several samples, and this significantly reduces the diagnostics cost for one patient. Many multi-genic panels are formed according to the genes belonging to one class of diseases rather than according to the principle of the united phenotype as it is. For example, some diagnostic NGS kits combine all the genes of hereditary cancer and are used to examine patients with cancer of the breast, ovary, colon, etc. [12–14]. NGS technology can identify new genes for hereditary cancer. In addition, the number of patients examined using multi-genic panels has significantly increased. These works revealed that, in general, NGS is more reliable and versatile than the "standard" DNA analysis methods. The scientific literature has described cases in which mutations were detected in patients who could not be diagnosed using other DNA testing methods. A key feature of NGS, in contrast to Sanger sequencing, is the ability to detect major mutations, such as deletions or exon duplication.

Currently, several commercial gene panels designed to detect hereditary cancer are used in clinical diagnostics. As a rule, they include genes with both high and medium penetrance. In addition, the developers of NGS kits offer the ability to diagnose not only genes with definitively proven medical significance but also recently identified candidate genes. This leads to some ambiguity in the interpretation of the results of multi-genic tests. For example, if the identification of inactivating mutations in the *BRCA1* or *BRCA2* genes does not cause difficulties in interpreting the results of NGS, then the identification of a mutation in the *BRIP1* gene can indicate both a diagnosis of hereditary breast cancer and a genetic defect with incompletely proven significance. Nevertheless, in the medium term, we can expect the creation of population screening systems to determine the carriage of mutations that predispose subjects to the development of cancer [4, 12, 15–17].

Another field of application for multi-genic tests in oncology is the analysis of somatic mutations in tumors. The number of known target genes associated with sensitivity to certain drugs is relatively small, and in the best-case scenario, it is measured by dozens, or by hundreds when using the most advanced criteria. The probability of detecting each individual event in each particular tumor is usually negligible; for example, mutations in the *EGFR* gene characteristic of lung carcinomas are found only in isolated cases of other types of neoplasms. However, if we combine all the gene mutations that are promising for the choice of treatment in a single pool and analyze all the patients, this includes a significant number of individuals whose target can be identified for therapy [18–23]. The development of multi-genic panels for the diagnostics of certain mutations is a sophisticated problem. A significant number of predictive mutations occur in translocations with varying breakpoints. As an example, rearrangements in the *ALK*, *ROS1*, *NTRK1*, *NTRK2*, *NTRK3*, etc. genes can be cited. To identify such events, the introns on the DNA matrix must be sequenced and the ribonucleic acid (RNA) sequences (cDNA) must be analyzed. The interpretation of the results is an even bigger problem. Sufficiently frequent muta-

tions, for example, intragenic deletions of exon 19 in the *EGFR* gene or substitutions in codon 600 of the *BRAF* gene, have obvious predictive significance, but many other events, particularly rare amino acid substitutions, are far from always associated with the characteristics of tumor drug sensitivity [4, 21–25]. In addition to the analysis of individual mutations, an approximate estimate of the total number of somatic events can be of some significance. Several somatic gene disorders are associated with increased antigenicity of the tumor and, therefore, with a higher likelihood of a response to immune therapy [26]. There are several services in the world that specialize in the NGS analysis of tumors. They both perform the NGS analysis and interpret its results. Works evaluating the clinical efficacy of therapy prescribed based on data from multi-genic sequencing have been published. In general, a similar approach can achieve a positive effect in individual patients [27–29].

## Monitoring the molecular profile of a tumor during treatment

Almost all clinical oncology is based on a single analysis of tumor tissue, which is performed at the very beginning of treatment. This approach is extremely vulnerable; modern studies have demonstrated that a tumor significantly modifies its characteristics during therapy. These changes critically influence the neoplasm's range of drug sensitivity. The mechanisms of acquired resistance to drug therapy are divided into two groups. There are general patterns of tumor adaptation to therapeutic effects. For example, ion channels can be activated in the transformed cells through the outer membrane of which the excretion of drugs occurs. In many tumors, in the course of therapy, the partial inactivation of apoptotic processes is noted, which reduces the neoplasm sensitivity to drug exposure [30, 31]. Another group of methods for the "habituation" of carcinomas to therapy involves reprogramming specific signaling pathways that the drug acts on. For example, the molecular target itself can undergo conformational modification, which the therapeutic inhibitor affects. Cells can become resistant to targeted therapy by activating collateral signaling cascades. In some cases, a tumor clone is formed that has lost its dependence on the driver mutation that initially played a key role in the neoplasm pathogenesis [30, 31]. The neoplasm evolution during the treatment process occurs unusually fast; sometimes the complete transformation of the carcinoma biological properties takes only a few weeks. Apparently, in many cases, a similar process is associated with the selection of preexisting cells that are resistant to therapy [32]. In other situations, adaptation to therapeutic effects is achieved through the emergence of epigenetic or new epigenetic events.

Regardless of the scenario in which the tumor escapes systemic treatment, it should be recognized that therapy cannot be based solely on the analysis of the primary neoplasm, and constantly monitoring the changes in time of the tumor properties throughout the entire history of the disease is extremely important [30]. It should be borne in mind that several algorithms currently exist for prescribing treatment, depending on changes in the molecular profile of the tumor. During endocrine or HER2–specific therapy for breast cancer, the status of the corresponding receptors may change, which makes it inappropriate to continue the targeted drug administration. Treatment with aromatase inhibitors sometimes leads to activation of the *HER2* oncogene through point mutations, and accordingly, effective drugs are required for such an isoform of the HER2 receptor [33, 34]. The treatment of lung tumors with gefitinib or erlotinib in approximately half of cases is accompanied by the appearance of a T790M mutation in the gene encoding the EGFR. To inactivate the T790M-mutated EGFR protein, a special drug, osimertinib, has been developed [35]. The most obvious example of a reanalysis of a tumor in the course of treatment is the study of surgical material obtained after neoadjuvant therapy. In this case, patients are not subjected to separate interventions aimed at obtaining representative fragments of the neoplasm. It is much more difficult to monitor the metastatic foci of carcinomas, which serve as an object for the impact of systemic therapy over long periods of time. In some cases, such as with targeted therapy for lung cancer, serial biopsies are acceptable [36–38]. These invasive procedures involve significant risks for the patient and place a high burden on the healthcare system as a whole. In most cases, the progression of the disease is accompanied by the emer-

gence of several metastatic foci, each of which may have its own path of molecular evolution. Performing serial biopsies of all tumor nodes seems an absolutely unacceptable scenario for examining a patient. Thus, significant efforts are aimed at developing methods for the non-invasive monitoring of neoplasm biological status [30].

The technology of the so-called fluid biopsy has gained great popularity. In cancer patients, peripheral blood and other fluids may contain tumor fragments, which are comprised of circulating transformed cells, tumor-specific (tissue-specific) proteins, DNA fragments, microRNAs, etc. The analysis of tumor-specific DNA sequences is the most promising technological platform for fluid biopsy since many molecular and genetic techniques (polymerase chain reaction, NGS, etc.) can identify a single mutated copy of a gene in an excess of normal DNA. Moreover, a serial analysis of plasma samples does not place undue burdens on the patient. Fluid biopsy is believed to provide an integral idea of the biological status of all tumor foci in the body [39–41]. Currently, fluid biopsy is already used in practical oncology to analyze the EGFR T790M mutations in patients receiving treatment with gefitinib, erlotinib, or afatinib. Based on the results of this test, a decision is made on the reasonability of prescribing a third-generation EGFR inhibitor, such as osimertinib [42, 43]. In the medium term, the application of fluid biopsy is expected to expand markedly.

## Personalized *ex vivo* tumor models

It is advisable to start this section with an example that shows modern approaches for the treatment of infectious diseases. Currently, inoculation is a routine method of examining patients with signs of infection. This procedure can not only establish the range of pathogenic microorganisms, but it can also assess their sensitivity to antibiotics. Approximately the same approach can be applied to patients with cancer. There are *ex vivo* methods for culturing tumor cells. Such technology, at least in theory, may allow a series of tests aimed at personalization of the antitumor therapy selection [44]. Obtaining *ex vivo* individual tumor clones is a rather complicated process. The simplest approach is the cultivation of tumor cells *in vitro*. In the vast majority of cases, these cells stop mitosis within a few passages; therefore, many tests are focused primarily on working with so-called short-term cultures. For individual patients, so-called long-term culture can be obtained; as a rule, this is due to the fact that additional genetic events occur during the passage of tumor cells, which ensures immortalization of the cell line. There are a huge number of different laboratory techniques to increase the efficiency of the process of obtaining cell cultures. They include the use of various nutrients, auxiliary growth factors, biochemical additives, substrates, etc. Nevertheless, many types of neoplasms are difficult to sub-inoculate into culture, and this limitation is typical for carcinomas with a relatively favorable course, in particular breast tumors, neoplasms of the prostate, etc. [44–47].

In developed countries, research programs have been developed that aim at obtaining xenografts from each patient with cancer. In this case, a tumor fragment is transplanted into immunodeficient mice. In general, the efficiency of obtaining tumor clones during inoculation in animals is higher compared to attempts to obtain cell cultures. This is apparently due to the fact that living organisms provide more favorable conditions for maintaining the viability of transplanted cells [46, 48, 49]. As a rule, tumor cell lines and xenografts retain driver mutations that caused the malignant transformation. Accordingly, individualized tumor models represent the ability of a primary tumor to respond, for example, to therapy with mutated kinase inhibitors. However, taking into account the more integrated biological characteristics of neoplasms, the question of the potential medical information content of individual *ex vivo* models remains unresolved. During inoculation, only a single clone of tumor cells "survives," which may not fully reflect all the aspects of the initial neoplasm. In addition, as mentioned above, a fairly large number of powerful biologically active substances are used in the process of growing tumor cells *ex vivo*. These manipulations certainly lead to modifications of many essential components of neoplasm life activity. It should be remembered that antitumor therapy acts when the immune system is functioning; it is not completely clear how manipulations with

cell cultures or immunodeficient mice can adequately indicate the characteristics of interactions between tumor cells and drugs in a living organism [44].

## Additional information

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